Qazvin University

Of Medical Sciences

Osteogenic Differentiation of MSCs

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Overview

Bone marrow properties

- Bone marrow is a highly cellular structure present within the hollow cavities of hard bone tissue

- There are 2 types of bone marrow:

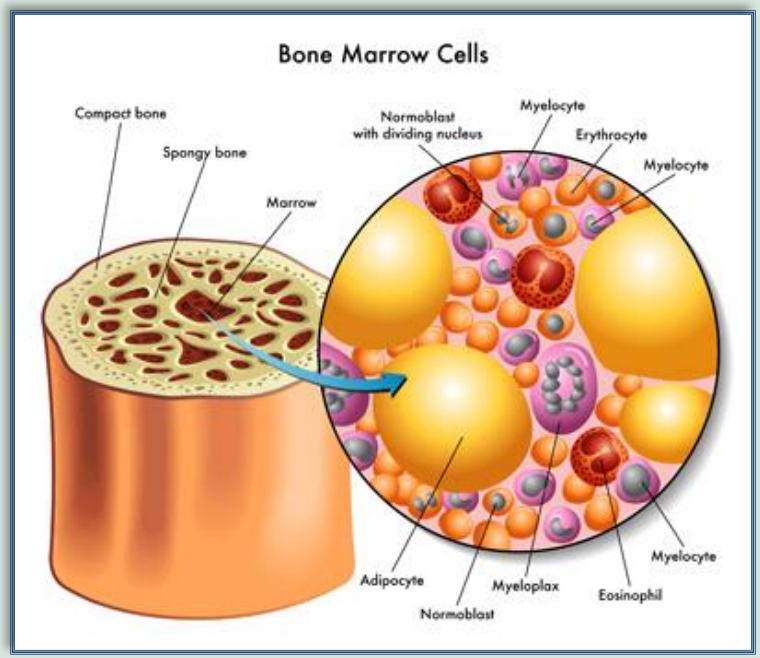
A) Red bone marrow

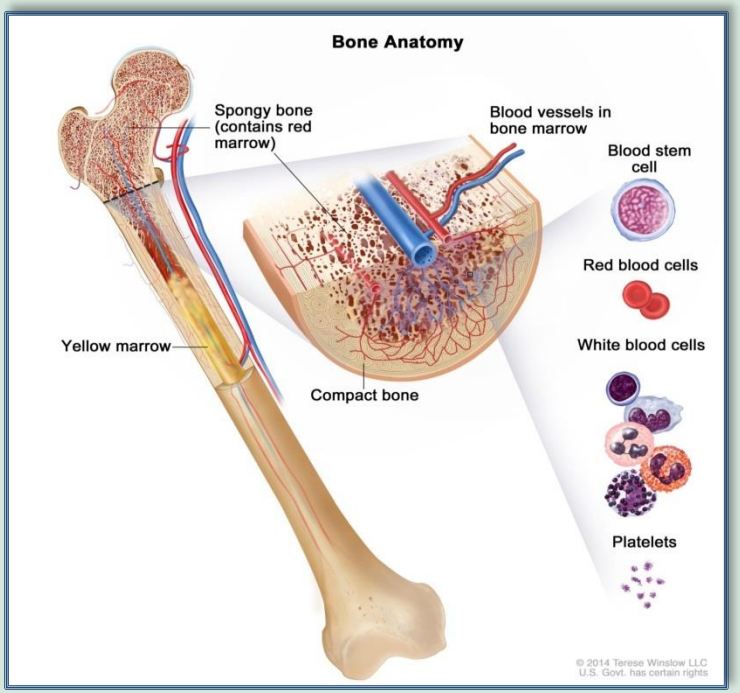
B) Yellow bone marrow

- **Children**: bone marrow in all bones is red bone marrow
- **Adulthood:** bone marrow cells in long bones of hand and leg become non-functional and are replaced by fat cells to form yellow bone marrow

- The only bone to carry red bone marrow throughout life are vertebrae, sternum, hip bone and skull bones

Constitutes 4% of the total body mass of human


Bone Marrow Cells
Compact bone
Spongy bone
Marrow
Normoblast with dividing nucleus
Myelocyte
Erythrocyte
Myelocyte
Myelocyte
Adipocyte
Myeloplax
Eosinophil
Normoblast


Bone Anatomy
Spongy bone (contains red marrow)
Blood vessels in bone marrow
Blood stem cell
Red blood cells
White blood cells
Platelets
Yellow marrow
Compact bone
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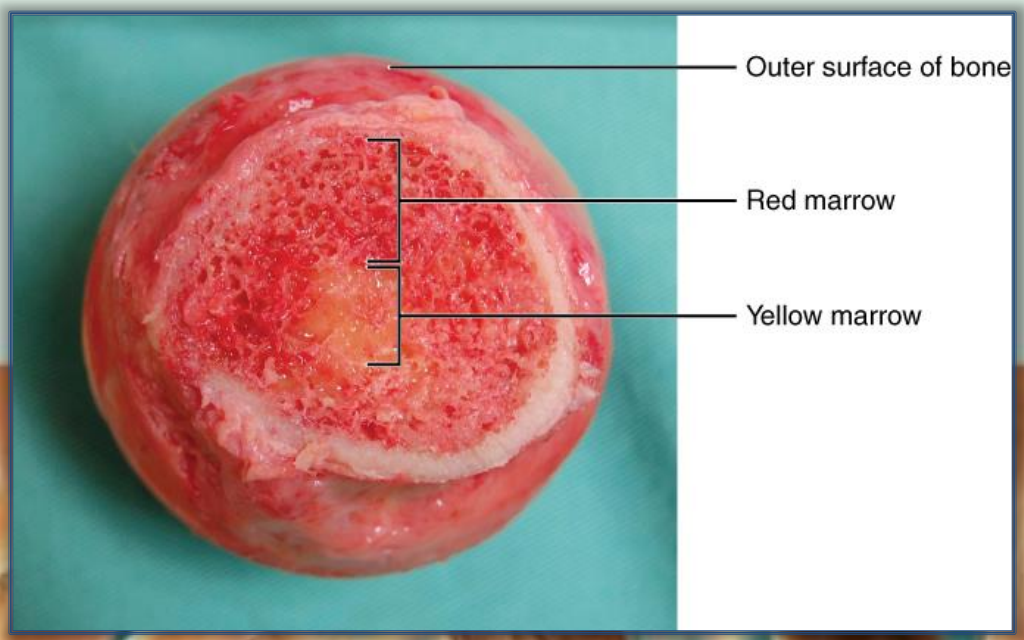

Outer surface of bone
Red marrow
Yellow marrow

- Bone marrow cells are highly functional and continuously divide and give rise to the different cells present in blood.

- Changes in bone marrow cause change in the composition of blood which can lead to various diseases. [1]

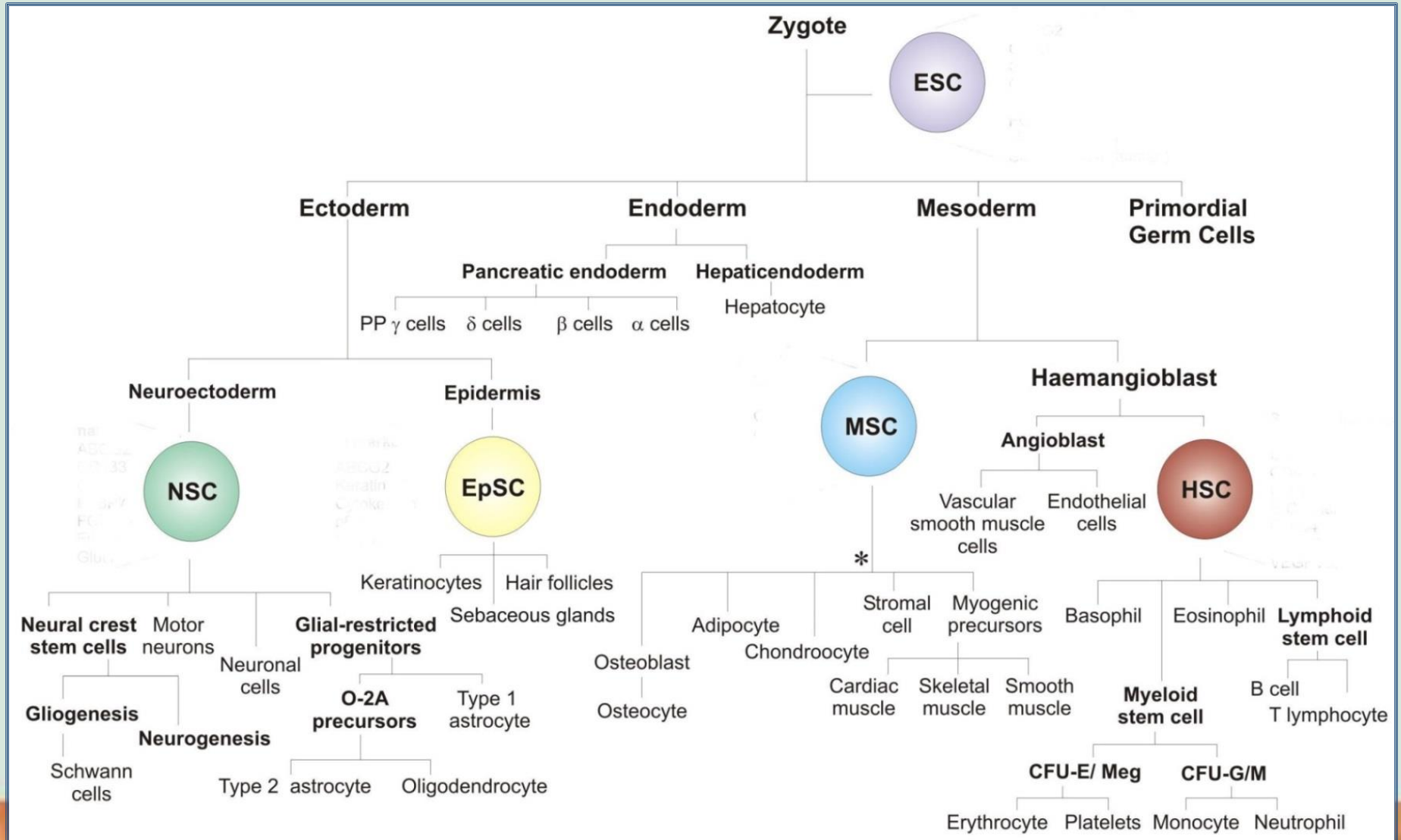
Stem Cells

What Are Stem Cells?

- An undifferentiated cell of a multicellular organism.
- Remarkable potential to develop into different cell types.
- Capable of giving rise to more cells of the same type.
- Other kinds of cell arise by differentiation.
- Capacity to self-renew.
- **Potency (Toti / Pluri / Multi / Unipotent)**[2]

Potency

- **Totipotent:** zygote / any cell / whole body / three layers
- **Pluripotent:** toti-derived / ESCs / not whole body / multipotent cells
- **Multipotent:** pluri-derived / ASCs (HSCs and MSCs)
- **Unipotent or Committed:** one type / self-renewal / tissue-specific stem cells[2,3]



Development of stem cells within the body. This is not a comprehensive diagram, for clarity only selected stem cells are shown . ESC = embryonic stem cell, NSC = neuronal stem cell, EpSC = epidermal stem cell, MSC = mesenchymal stem cell, HSC = haematopoeitic stem cell. *Differentiation of MSCs along neuronal lineages has also been demonstrated, see text for information. Modified from R&D Systems website (http://www.rndsystems.com). Copyright BTR©

- Non-hematopoietic multipotent cells in the stromal compartment of the bone marrow were first identified by Friedenstein and colleagues.

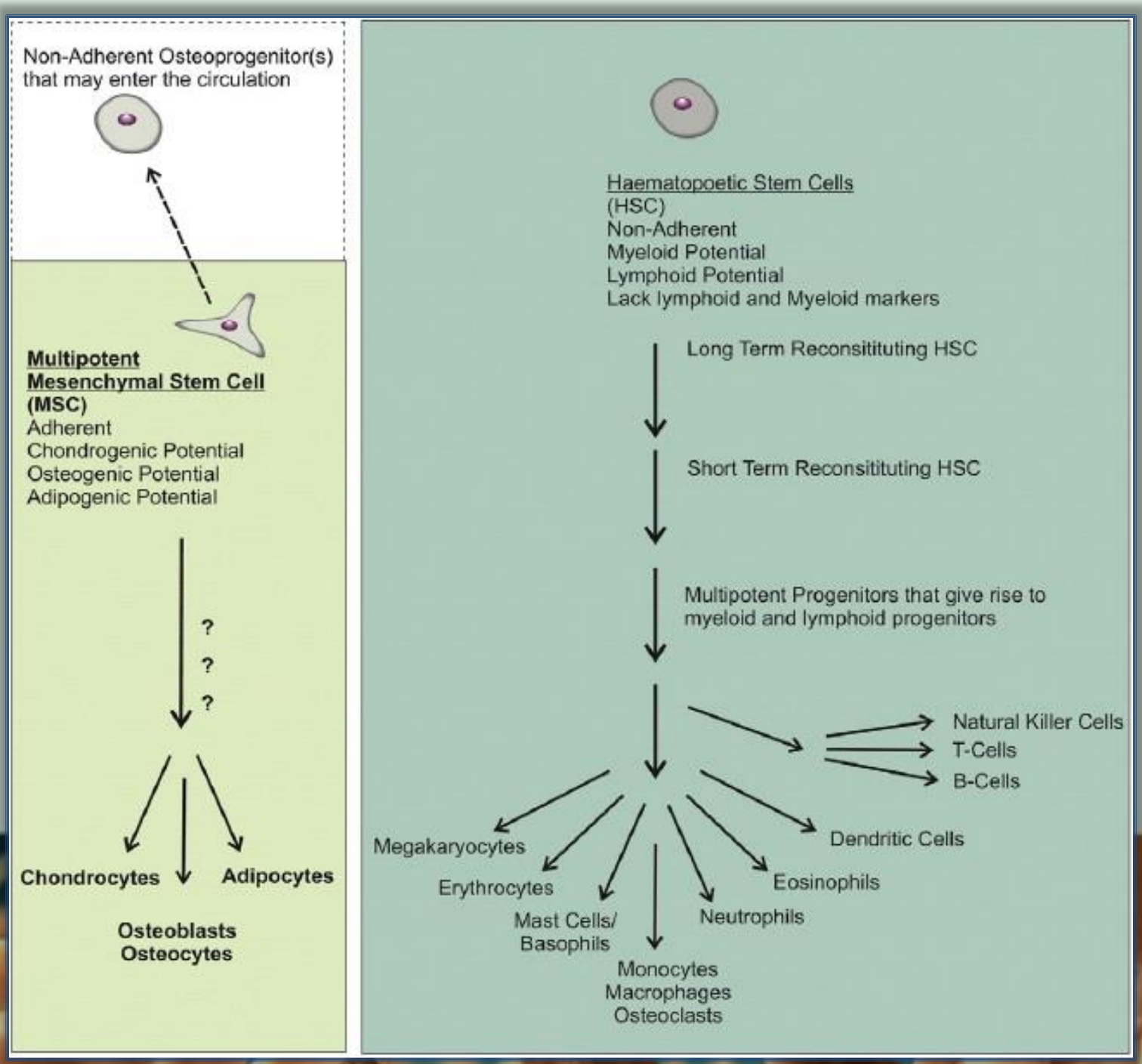
- Have high proliferative potential and the ability to differentiate into chondrocytes, osteoblasts, adipocytes, and stromal cells that support hematopoiesis . Furthermore, they have immunomodulatory activity

Criteria for the identification of MSCs from International Society for Cellular Therapy (ISCT):

(1) Adherence to plastic

(2) Differentiation into chondrocytes, osteoblasts, and adipocytes under standard in vitro differentiating conditions

(3) Expression of surface markers CD105, CD73, and CD90, in the absence of CD45, CD34, CD14, CD11b, CD79α, CD19, and HLA-DR [4]


Non-Adherent Osteoprogenitor(s) that may enter the circulation
Multipotent Mesenchymal Stem Cell (MSC)
Adherent
Chondrogenic Potential
Osteogenic Potential
Adipogenic Potential
? ? ?
Chondrocytes
Adipocytes
Osteoblasts
Osteocytes
Haematopoetic Stem Cells
(HSC)
Non-Adherent
Myeloid Potential
Lymphoid Potential
Lack lymphoid and Myeloid markers
Long Term Reconsitituting HSC
Short Term Reconsitituting HSC
Multipotent Progenitors that give rise to myeloid and lymphoid progenitors
Natural Killer Cells
T-Cells
B-Cells
Dendritic Cells
Eosinophils
Neutrophils
Megakaryocytes
Erythrocytes
Mast Cells/ Basophils
Monocytes Macrophages Osteoclasts

BMSCs may be an important MSC population to consider for three reasons.

First: their activity supports another distinct population of progenitor cells that give rise to blood cells and immune system: hematopoietic progenitors .

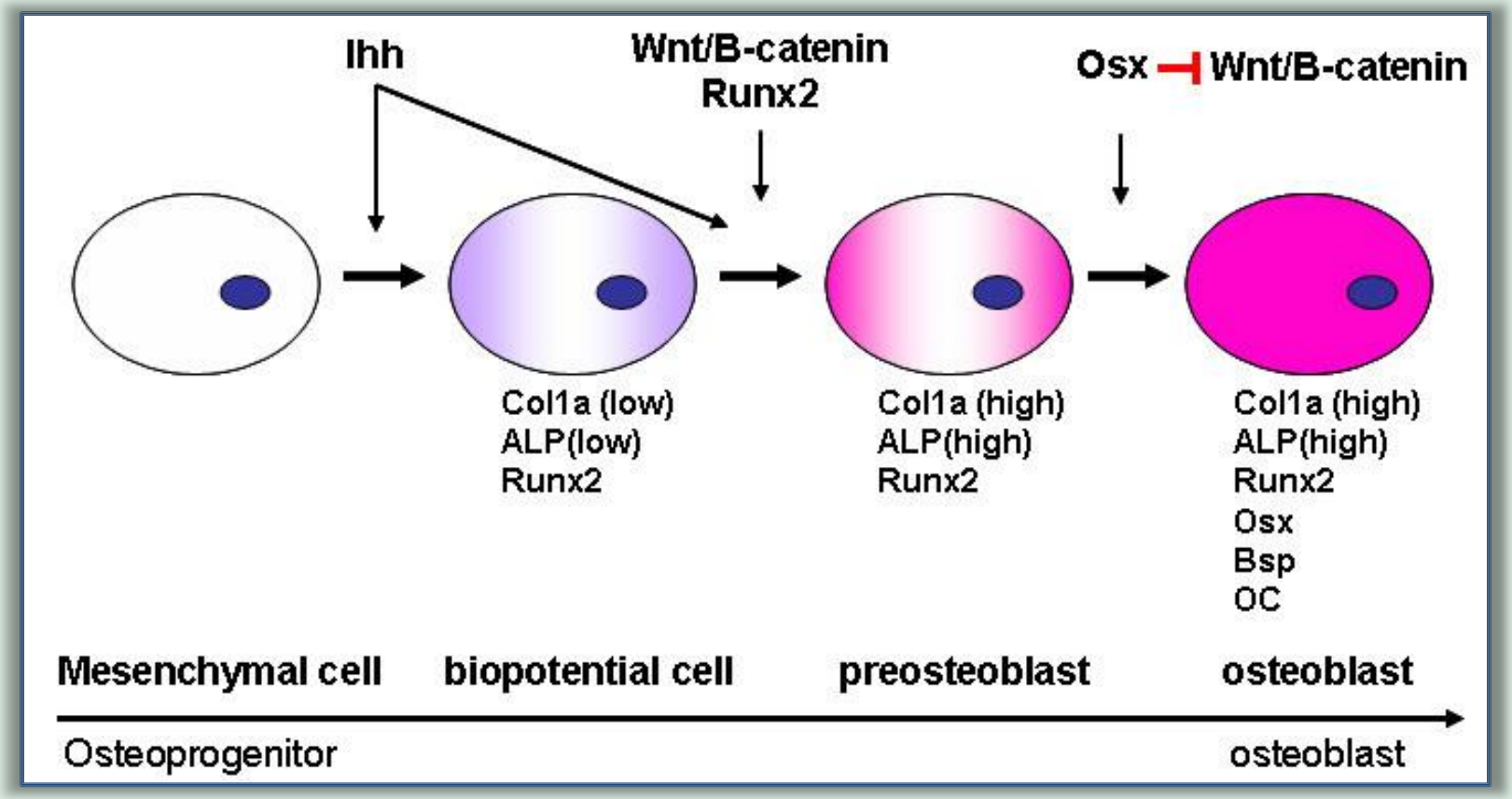
Second: BMSCs could be used to encourage repair of extramedullary tissues

Third: BMSCs may influence bone growth and remodeling

OSTEOGENIC DIFFERENTIATION OF MSCS *IN VITRO*

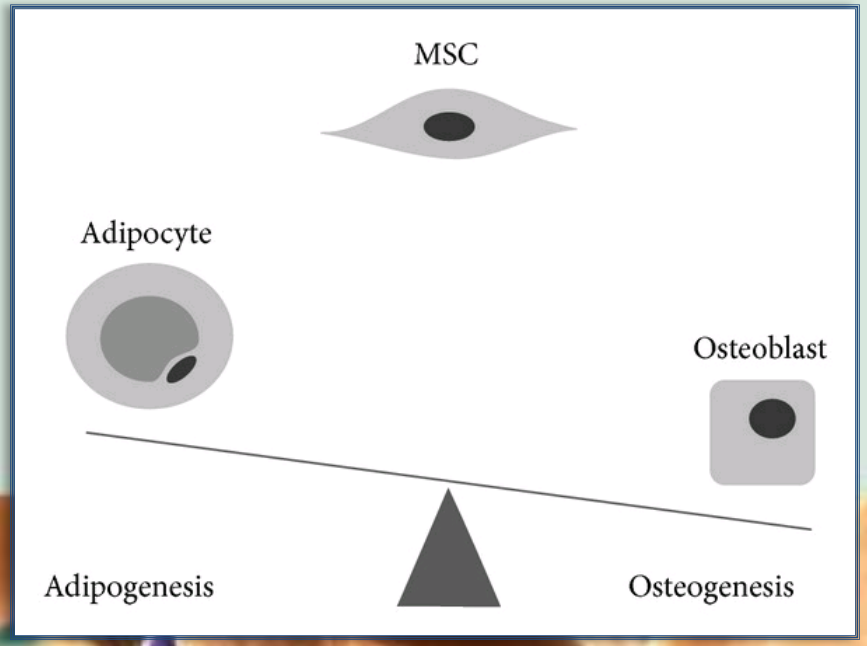
It has been divided into three stages :

- **Days 1 to 4 :peak in the number of cells** is seen.
- **Days 5 to 14:** characterized by the transcription and protein **expression of alkaline phosphatase (ALP).** After this initial peak of ALP its level starts to decline. Also found at an early stage is the **expression of a collagen type I matrix** onto which the mineral is deposited.
- **Days 14 to 28: high expression of osteocalcin and osteopontin**, followed by calcium and phosphate deposition. [5]


Ihh
Wnt/B-catenin
Runx2
Osx
Wnt/B-catenin
Col1a (low)
ALP(low)
Runx2
Col1a (high)
ALP(high)
Runx2
Col1a (high)
ALP(high)
Runx2
Osx
Bsp
OC
Mesenchymal cell
biopotential cell
preosteoblast
osteoblast
Osteoprogenitor
osteoblast

While MSC differentiation can be directed by multiple microenvironmental factors (such as mechanical forces , electrical currents , and magnetic fields), but we will specifically focus on **cytokine signaling** that govern MSC lineage differentiation.

The commitment and differentiation of MSC towards an adipogenic or osteogenic cell fate depend on a variety of signaling and transcription factors. A large body of experimental evidence suggests that an **inverse correlation** exists between **adipogenesis and osteogenesis** [6]



Factors involved in determination of BMSCs commitment into Osteoblast [9]

Gene name	Gene symbol	Osteoblast differentiation
Canonical Wnt signaling	Wnt3a/Wnt10b	↑
Non-canonical Wnt signaling	Wnt5a	↑
Bone morphogenetic protein	BMP2	↑
Sonic hedgehog	Shh	↑
Transforming growth factor-β1	TGF-β1	↑
Nel-like molecule, type 1	Nell-1	↑
WW domain containing transcription regulator 1	Wwtr1	↑
Sprouty homolog 1 (*Drosophila*)	Spry1	↑
Retinoblastoma 1	Rb1	↑
Myocyte enhancer factor-2 interacting transcriptional repressor/histone deacetylase 9c	MITR/HDAC9c	↑
Nuclear factor I–C	NFI-C	↑
Lysine (K)-specific demethylase 6A	KDM6A	↑
Bmi1 polycomb ring finger oncogene	Bmi1	↑
Msh homeobox 2	Msx2	↑
Transcriptional co-activator with PDZ-binding motif	TAZ	↑
Heme oxygenase (HO)-1	HO-1	↑
Secreted protein acidic and rich in cysteine	SPARC	↑
Farnesoid X receptor	FXR	↑
NAD-dependent deacetylase sirtuin-1	Sirt1	↑
Peroxisome proliferator-activated receptor-γ	Ppar	↓
Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	SERPINF1	↓
SRY (sex determining region Y)-box 2	Sox2	↓
Enhancer of zeste 2 polycomb repressive complex 2 subunit	EZH2	↓
Transducing-like enhancer of split 3	TLE3	↓
S100 calcium binding protein A16	S100A16	↓
Neuropeptide Y (NPY) receptor 1	Y1 receptor	↓
Thymosin, beta 4, X chromosome	Tmsb4x	↓
Chemerin/cognate receptors CMKLR1	Chemerin/CMKLR1	↓

Several cell signaling cascades exemplify proosteogenic/ antiadipocytic stimuli that include:

- **β-catenin dependent Wnt signaling** (as well as β-catenin independent signaling)
- **Hedgehog signaling**
- **NELL-1** (NEL-like protein 1) **signaling**

Dissimilarly, various signaling cascades demonstrate positive regulation of both osteogenesis and adipogenesis

- **Bone morphogenetic proteins (BMPs):**While the majority of BMPs promotes osteogenic commitment and differentiation of MSC BMPs also demonstrate proadipogenic effects

- **Insulin like growth factor (IGF) signaling** : likewise demonstrates dual proosteogenic/proadipogenic effects

Control of Adipogenesis and Osteogenesis by Transcription Factor

Signaling cascades which promote MSC osteogenic and/or adipogenic lineage differentiation generally converge on two key transcription factors

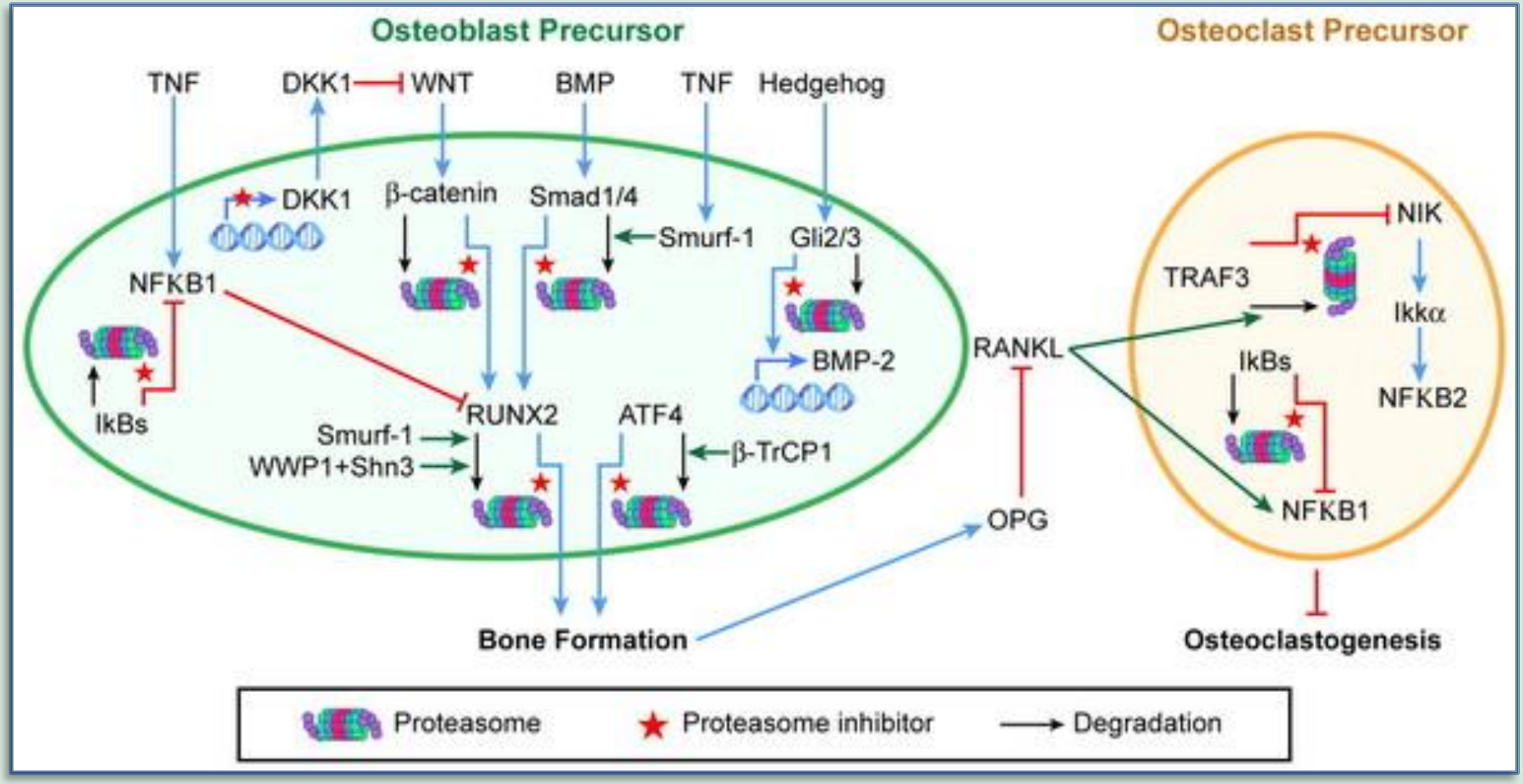
- **Runx2**:The Master Osteogenic Transcription Factor
- **PPARγ**:The Master Adipogenic Transcription Factor

Runx2

In regard to osteogenic differentiation, Runx2 activates and regulates osteogenesis as the targeted gene of many signaling pathways, including but not limited to transforming growth factor-beta 1 (TGF-β1),BMP, Wingless type (Wnt), Hedgehog (HH), and (Nel)-like protein type 1 (NELL-1)

PPARγ

- Peroxisome proliferator-activated receptors are members of the steroid/thyroid hormone receptor gene superfamily
- All three PPARs are found in mammals and are activated by poly unsaturate fatty acids
- PPARγ is principally regarded as the master regulator of adipogenesis, for no other factor can rescue adipocyte formation in the event of PPARγ knockout, and generally all proadipogenic cell signaling pathways converge with PPARγ[7]



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The identification of osteoblasts

- Cell morphology
- Expression of type I collagen
- Alkaline phosphatase (ALP) activity
- The mRNA expression of OCN

Cell morphology and cytochemical characteristics

- Adhere to the bottom of the culture dish after 4 to 24 h. On day 3: possess typical shapes, arranged linearly in a stepping-stone arrangement

- Cell numbers increased gradually and reached confluence on day6. Hematoxylin-eosin staining images showed that the nuclei were stained an amethyst color and the cytoplasm was stained pink Cell proliferation was determined with a kit

- Calcification nod staining was investigated by the Alizarin RedS staining method.Alizarin red calcium deposition was measured at day 21.Mineralized matrix, indicated by bright red staining was identified by light microscopy.

Alkaline phosphatase (ALP) activity

- ALP activity was increased when co-cultured with osteoblasts, compared with negative control group
- ALP activity in HUMSCs was determined at 7, 10 and 14 days of incubation using alkaline phosphatase activity colorimetric assay kit

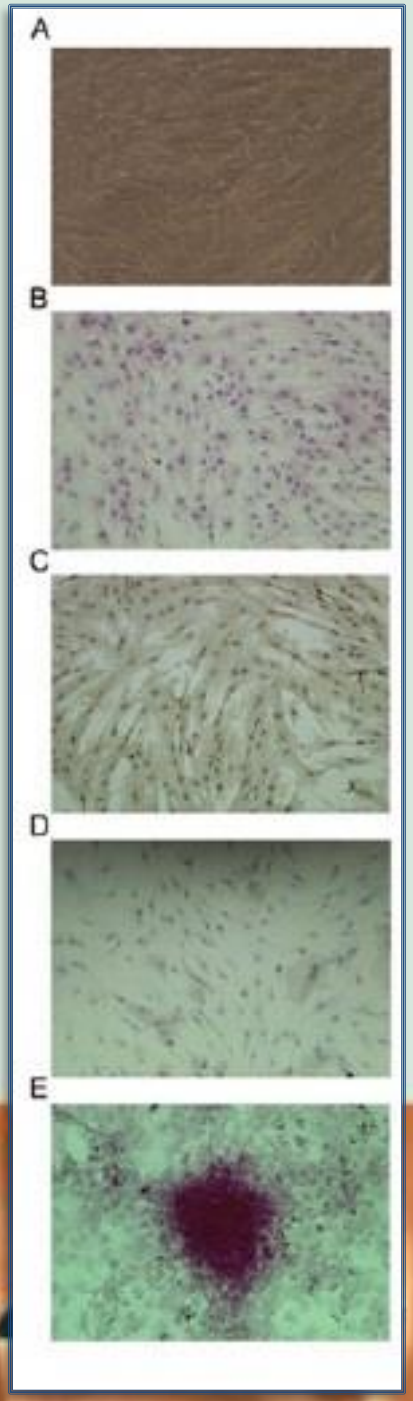
The expression of type I collagen protein

In the HUMSC co-cultured with different osteoblasts for 10 days, the human-specific type I collagen antibody clearly revealed type I collagen protein expression. The expression of type I collagen protein was indicated by bright red fluorescence, cell nuclear staining was indicated by blue fluorescence.

The mRNA expression of OCN

HUMSCs co-culture with the osteoblasts induced the expression of OCN, which were nearly undetectable in the negative control group.

To assess the osteogenesis of HUMSCs, osteocalcin(OCN) mRNA expression was detected by real time RT-PCR 21days after cell co-culture [8]



A: Morphology of osteoblasts

B: Hematoxylin-eosin staining

C: Expression of type I collagen

D: ALP staining

E: Alizarin Red S staining

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Thanks for your attention

Questions & Ask